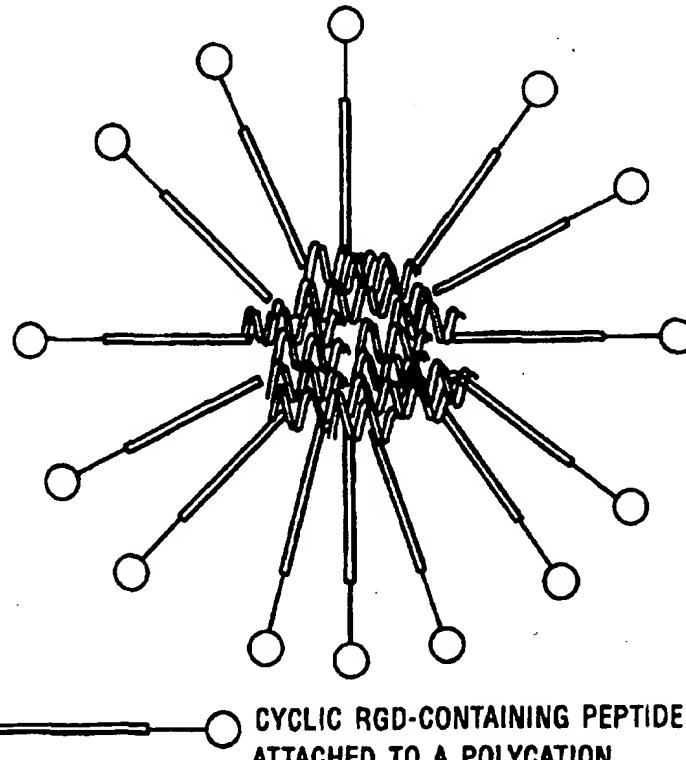




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<p>(21) International Application Number: <b>PCT/GB95/02706</b></p> <p>(22) International Filing Date: 17 November 1995 (17.11.95)</p> <p>(30) Priority Data:</p> <table> <tr> <td>9423231.1</td> <td>17 November 1994 (17.11.94)</td> <td>GB</td> </tr> <tr> <td>9423308.7</td> <td>18 November 1994 (18.11.94)</td> <td>GB</td> </tr> <tr> <td>9512822.9</td> <td>23 June 1995 (23.06.95)</td> <td>GB</td> </tr> </table> <p>(71) Applicant (for all designated States except US): IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY &amp; MEDICINE [GB/GB]; Sherfield Building, Imperial College, London SW7 2AZ (GB).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): HART, Stephen, Lewis [GB/GB]; Division of Cell and Molecular Biology, Institute of Child Health, 30 Guilford Street, London WC1N 1EH (GB). HARBOTTLE, Richard, Paul [GB/GB]; St. Mary's Hospital Medical School, Norfolk Place, London W2 1PG (GB).</p> <p>(74) Agents: CHAPMAN, Paul, William et al.; Kilburn &amp; Strode, 30 John Street, London WC1N 2DD (GB).</p>		9423231.1	17 November 1994 (17.11.94)	GB	9423308.7	18 November 1994 (18.11.94)	GB	9512822.9	23 June 1995 (23.06.95)	GB	<p>(81) Designated States: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG).</p> <p>Published With international search report.</p>	
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<p>(54) Title: INTERNALISATION OF DNA, USING CONJUGATES OF POLY-L-LYSINE AND AN INTEGRIN RECEPTOR LIGAND</p> <p>(57) Abstract</p> <p>Compositions comprising DNA associated with a polycation moiety wherein the polycation moiety is itself coupled to an integrin receptor binding moiety are disclosed. Preferably, the integrin receptor binding moiety is a peptide, and the compositions can be used to deliver DNA to a cell where it will be expressed, for example, to treat a condition by gene therapy. In a preferred embodiment, the integrin receptor binding moiety comprises a peptide, in particular a cyclic peptide, comprising the sequence RGD. In a particularly preferred embodiment, the peptide comprises the sequence GGCRGDMFGC. Cyclic configuration in this sequence is imposed by virtue of the presence of two cysteine residues which can form a disulphide bond.</p> 												

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INTERNALISATION OF DNA, USING CONJUGATES OF POLY-L-LYSINE AND AN INTEGRIN RECEPTOR LIGAND

The present invention relates to compositions comprising DNA associated with a polycation entity which is itself linked to an integrin specific binding moiety. The compositions of the invention can be used to deliver DNA to cells for internalisation and expression therein. In particular, therefore, the invention relates to methods of obtaining gene expression in cells to overcome genetic deficiencies.

In recent years, with the continuing identification of specific genes responsible for certain disease conditions, the concept of "gene therapy" has attracted a great deal of attention. The potential to deliver a new gene, or even part of a gene sequence, to a defective cell in order to correct such an inherent deficiency is an attractive one. There are, of course, inherent problems in such an approach. For instance, the DNA must be delivered in a form that will be taken up, or internalised, by the target cell. Furthermore, the DNA itself must be expressed effectively in the cell in order to overcome the genetic deficiency. Inherent in these problems is the additional one that the DNA itself, after having entered the cell, must be protected in some way to prevent its damage, or even destruction, by, for example, cellular enzymes.

One potential approach to this problem of internalisation, and protection, of DNA is disclosed in Hart et al (J. Biol. Chem., 269, No 176: 12468-12474 (1994)). This approach exploits the presence of integrin receptors on cell surfaces for achieving internalisation of filamentous phage. Integrins are a super family of

heterodimeric cell adhesion molecules that consist of several different  $\alpha$  and  $\beta$  subunits. Their cellular function is to mediate the movement, shape and polarity of cells through binding with proteins of the extracellular matrix. In addition, integrins are exploited as receptors for cell entry by pathogenic bacteria, such as *Yersinia pseudotuberculosis* (Isberg, R., *Science*, 252: 934-938 (1991)) and *Bordatella pertussis* (Relamna et al, *Cell*, 61: 1375-1382 (1990)).

10

Hart et al (supra) found that displaying an integrin-binding peptide sequence on the surface of bacteriophage fD particles enabled the phage particles to be internalised by mammalian cells. However, no effective expression of DNA carried by phage particles was shown.

In addition, there are certain problems associated with the use of such particles to deliver DNA in this fashion. Firstly, there is a packaging size limitation governed by the size of the phage particle itself. Only genetic material up to a particular particle size could be delivered in this fashion. Secondly, the phage itself will only package single stranded genetic material, and this would not be effectively expressed in a mammalian cell system. Finally, the phage itself consists of other proteins and is a somewhat "messy" system for delivering DNA. It is possible that these additional components would have a material effect on whether or not genetic material was expressed.

20

Other approaches to delivering DNA into mammalian cells are disclosed in WO-A-9418834. Here, DNA was conjugated with a polyelectrolyte to form a complex which was then inserted into an embryonic cell, a germ cell or a germ

5 precursor cell. This method was disclosed primarily for producing transgenic animals. The methods disclosed in this document rely on either microinjection of the complex directly into the germ cell, or by having the polycation/DNA complex present in the culture medium and relying on uptake by the cells.

10 Cotton et al (PNAS USA, 87: 4033-4037 (1990)) used the natural iron-delivery protein transferrin, coupled to DNA binding polycations such as polylysine or protamine, to deliver DNA into human leukaemic cells. However, they also found that they required the use of other agents to effect the survival of the transfected DNA or to modulate 15 transferrin receptor levels so as to increase the internalisation or uptake of the DNA itself. These steps included increasing the transferrin receptor density through treatment of the cells with the cell-permeable ion chelator, desferrioxamine, interfering with the synthesis of heme with succinol acetone treatment or 20 stimulating the degradation of heme with cobalt chloride treatment. In other words, effective uptake and expression of the DNA could only be achieved through the use of "co-factors" or co-treatments.

25 Thus, there exists a need for further and better methods of delivering DNA to a cell such that it will be internalised and expressed efficiently therein, preferably without the need for any other co-factors or co-treatments, and in a form which is not limited to 30 genetic material of a particular size.

The approach taken by the present inventors is to use specific cell-surface integrin receptor binding moieties coupled to a polycation moiety which will bind to DNA.

The "DNA packages" will then bind to cell surface receptors and be internalised. It is also surprisingly been found that such an approach results in efficient expression of DNA so internalised, without the need for 5 any co-factors or co-treatment. Nevertheless, co-factors can be used where desired. A preferred co-factor is chloroquine or any other factor which reduces endosomal degradative activity. The observation of improved expression in the presence of chloroquine may be because 10 the peptide-DNA complex is internalised, at least in part, to endosomal compartments. Chloroquine is a weak buffer which is purported to prevent acidification of endosomal vesicles which limits the activity of endosomal degradative enzymes. Thus the internalised peptide-DNA 15 complex has more opportunity to escape the endosome and avoid degradation. Other factors which might have a similar beneficial effect include ammonium chloride, another weak buffer which works like chloroquine; fusogenic peptides related to the N-terminus of the HA 20 protein of influenza virus which mediate active membrane disruption and inactivated adenovirus capsids which also disrupt the membrane of the endosome.

25 One advantage of integrin receptor mediated internalisation is that large particles can be internalised, e.g. whole cells.

30 Thus, in a first aspect, the present invention provides a composition comprising DNA associated with a polycation wherein the polycation is coupled to an integrin receptor binding moiety.

In the present invention, "DNA" means single or double stranded DNA, either as complete coding sequences or

parts thereof and, in particular, refers to coding sequences for one or more genes.

5 "Integrin receptor binding moiety" means any moiety or species capable of specifically binding to integrin receptors found on the surface of cells. In particular, it refers to integrin receptor binding peptides capable of binding to integrin receptors.

10 "Association" of the DNA and the polycation occurs, for example, by virtue of charge-charge interaction, but other forms of association are equally applicable.

15 In a preferred embodiment, the integrin receptor binding moiety comprises a peptide, in particular a cyclic peptide, comprising the sequence RGD. In a particularly preferred embodiment, the peptide comprises the sequence GGCRGDMFGC. Cyclic configuration in this sequence is imposed by virtue of the presence of two cysteine 20 residues which can form a disulphide bond.

25 The compositions of the present invention bind effectively to integrin receptors found on cell surfaces and are internalised. The DNA is then effectively expressed by the cell without the need for any other co-factors being present or the need for any co-treatment. Of course, co-factors or co-treatments can be used in conjunction with the present invention to boost expression levels even further.

30 The polycation moiety can be any suitable polycation capable of forming a complex with DNA. In particular, polycations such as polylysine can be used. The number of residues in the polycation can vary from a relatively

small number, up to quite long chains, or can be a mixture thereof. For example, polycations of from 3-1000, 3-500 or indeed 3-100 residues can be used. In particular, 10-16 cation residues are suitable, 5 particularly 16. In one embodiment of the invention, therefore, the polycation consists of 10-16 polylysine residues, with 16 lysine residues being particularly preferred.

10 Without in any way being limited, it is believed that the polycation "tails" of the compositions of the invention associate with the DNA to be delivered, effectively forming a "package" with the integrin receptor binding moieties on the outside. The DNA composition can then 15 bind to an integrin receptor on the cell surface and be internalised. The polycation may then act to protect the DNA from the cell's internal enzyme systems, enabling it to be integrated in the cell's genome and thus expressed.

20 In a further aspect, the present invention provides a DNA binding composition comprising an integrin receptor binding moiety coupled to a polycation. Preferably the integrin binding moiety is a peptide, as described above. Here coupling may occur to the C-terminus or to the N- 25 terminus of the peptide. In one preferred embodiment, the polycation is polylysine.

30 This composition can then simply be brought into contact with DNA to "package" the DNA for delivery to a designated cell.

As discussed herein, compositions of the present invention enable the effective delivery of DNA to cells wherein it is internalised and expressed efficiently. In

5 this way, genetic deficiencies of particular cell types can be overcome by the delivery and expression of DNA sequences encoding correct or "native" proteins. One example where such an approach may be effective is in the treatment of cystic fibrosis. Thus, in other aspects, the present invention provides:

10 (a) the use of a composition of the invention in the manufacture of a medicament for the treatment or prophylaxis of a condition related to a genetic deficiency or modification;

15 (b) a composition of the invention for use in the treatment or prophylaxis of a condition related to a genetic deficiency or modification;

20 (c) a method for the treatment or prophylaxis of a condition related to a genetic deficiency comprising the step of administering to a subject a composition of the invention;

25 (d) a method for the transformation of a host cell comprising the step of bringing together the cell with a composition of the invention; such methods find use generally in transfection of cells, particularly mammalian cells;

30 (e) the use of a composition of the invention in the preparation of a medicament for the treatment or prophylaxis of a condition caused by a genetic deficiency or modification; and

(f) a pharmaceutical formulation comprising a composition of the invention together with one or

more pharmaceutically acceptable carriers, diluents or excipients.

Preferred features of each aspect of the invention are as 5 for each other aspect *mutatis mutandis*.

The invention will now be described by reference to the following examples, which should not in anyway be construed as limiting the invention.

10

The examples refer to the figures in which:

15

FIGURE 1: shows a possible structure for the polycation-integrin receptor binding moiety/DNA complex.

20

FIGURE 2: shows levels of expression of a luciferase reporter protein in cells transformed with a composition of the invention, compared to suitable controls.

25

FIGURE 3: also shows levels of expression of a luciferase reporter protein in cells with a composition of the present invention, compared to suitable controls, but here the composition is different from that used in respect of Figure 2.

30

FIGURE 4: shows levels of expression of a luciferase reporter protein in cells transformed with various compositions of the invention;

FIGURE 5: shows the effect of chloroquine on expression of a luciferase reporter protein in COS-7 cells;

FIGURE 6: shows the effect of chloroquine on expression of a luciferase reporter protein in endothelial cells;

5 FIGURE 7: shows the results of expression of a luciferase reporter protein in COS-7 cells using differing ratios of RGD peptide:DNA, in the presence and absence of chloroquine;

10 FIGURE 8: shows the results of expression of a luciferase reporter protein in endothelial cells using differing ratios of RGD peptide:DNA, in the presence and absence of chloroquine; and

15 FIGURE 9: shows the results of comparing transfection of endothelial cells with varying ratios of K10 peptide:DNA and of K16 peptide:DNA.

20 **EXAMPLE 1:** Preparation of a first Polylysine-Integrin receptor binding peptide.

The peptide sequence GGCRGDMFGC(K)<sub>16</sub> was synthesised as follows:

25 (a) the peptide was synthesised on ABI model 431A solid-phase batch peptide synthesiser using Wang HMP resin and Fmoc-cleavage strategy;

30 (b) the linear peptide was cleaved from the resin using 5 ml of a scavenger mixture (0.75 g phenol, 0.25 ml EDT, 0.5 ml thioanisole, 0.5 ml deionised H<sub>2</sub>O, 10 ml TFA), the mixture was stirred for 2 hrs at room temperature and was then filtered over sinter

into ice-cold MTBE. This was then stored at -18°C before being spun down, washed with 3 x 6 ml MTBE before being dried in vacuo and redissolved in H<sub>2</sub>O and freeze dried;

5

10 (c) cyclisation of the peptide was carried out in 5% 'AcOH/20% DMSO v/v, buffered to pH 6 by 0.88 NH<sub>3</sub>(aq), with stirring for 24 hrs at room temperature. Finally, it was diluted (x 3) using deionised water;

15 (d) purification by ion-exchange chromatography was carried out using Mono-S resin, 50 mM HEPES buffer (pH 7.6) on Pharmacia FPLC system (monitored at 280 nm/UV-Hg lamp);

20 (e) fractions were assayed for effects on cell cultures;

(f) positive fractions were desalted using P2 Biogel and 0.1% aq TFA; and

25 (g) further desalting was carried out using reverse-phase chromatography and 0.1% aq TFA on an FPLC system (monitored at 214 nm/Zn lamp).

30 EXAMPLE 2: Internalisation and expression of a reporter gene using the polylysine-integrin receptor binding peptide described in Example 1.

5 µg of a luciferase reporter gene plasmid (pGL2 promega) was complexed with either the RGD-polylysine construct

(possible structure of complex is shown in Figure 1) or an equal concentration of polylysine in 100  $\mu$ l of Optimem media (Gibco). The DNA/peptide complexes and also a DNA only control were then applied to 50% confluent cultured (Caco-2) colonic epithelial cells which were then allowed to express for 48 hours. The cells were then harvested and the cellular protein analysed for luciferase activity (Relative Light Units). The activity shown in Figure 2 is adjusted to represent activity from 1 mg of cellular protein. In Figure 2 the term "RGD peptide" is used to indicate the polylysine-integrin receptor binding protein described in Example 1.

EXAMPLE 3: Preparation of a second polylysine-integrin receptor binding peptide.

The peptide sequence (K)<sub>16</sub>GGCRGDMFGCA was synthesised. This can be done using analogous techniques to those used in respect of Example 1. This peptide has a similar sequence to that referred to in Example 1, the main difference being that the polylysine region was present at the N-terminus rather than the C-terminus.

EXAMPLE 4: Internalisation and expression of reporter gene using the polylysine-integrin receptor binding peptide described in Example 3

The procedure described in Example 2 was repeated but using the polylysine-integrin receptor binding peptide described in Example 3 instead of the polylysine-integrin receptor binding peptide described in Example 1. Control experiments were also performed. The results are shown in Figure 3, wherein:

	DNA control	=	5 µg of PGL2 plasmid DNA
	PolyK	=	poly-L-lysine
5	RGD	=	newly synthesised RGD-containing peptide linked to polylysine (described in Example 3)
	Chlor	=	100 µM chloroquine
10	2 x RGD+DNA	=	twice as much as peptide, DNA same (5 µg)
	5 x RGD+DNA	=	five times as much peptide.

An indirect comparison of optimal peptide-DNA transfection efficiencies in the absence of chloroquine suggests that the peptide prepared in Example 3 ( $\pm 6 \times 10^7$  RLU/mg) is more efficient than the peptide prepared in Example 1 ( $\pm 3.5 \times 10^5$  RLU/mg in Figure 2) at delivery and expression of the luciferase reporter gene. The addition of chloroquine improved expression a further 2 fold approximately, suggesting that endosomal degradation is limiting the expression levels somewhat. It is interesting in comparison, however, that, in some circumstances, the efficiency of the transferrin-polylysine receptor-mediated gene delivery system was improved by more than 1,000-fold in the presence of chloroquine. The high level of expression by the RGD-polylysine peptide without co-factors and the relatively small improvement in enhancement with chloroquine is surprising.

30      EXAMPLE 5:      Transfection of Caco-2 cells with K16 RGD peptide and effect of chloroquine.

The polylysine-integrin receptor binding peptide of Example 1 was used in a repeat of the procedure of

Example 2, but using 1  $\mu$ g of luciferase reporter gene plasmid. The effect of chloroquine was also investigated. The results are shown in Figure 4. Highest transfection levels were achieved with 2 x RGD peptide and 100 nm chloroquine, these transfection levels being approximately 10-fold lower than those achieved with lipofectamine. It can again be seen that chloroquine gave a relatively small improvement with 2 x RGD peptide + DNA, although with RGD-peptide + DNA the improvement was greater.

**EXAMPLE 6:** Effects of chloroquine on transfection of COS-7 cells with RGD peptides.

The polylysine-integrin receptor binding peptide of Example 1 was again used. Essentially the methodology of Example 2 was followed with the substitution of COS-7 cells, and the use of different concentrations of chloroquine. The results are shown in Figure 5. It can be seen that 200  $\mu$ m chloroquine gave a 4-fold increase of expression, compared with no chloroquine. However, at these levels, cytopathic effects of chloroquine were apparent.

**EXAMPLE 7:** Effects of chloroquine on transfection of endothelial cells with RGD peptides.

This was a repeat of Example 6 using ECV 304 endothelial cells. The results are shown in Figure 6. For these cells, the highest level of expression was obtained in the absence of chloroquine. Although increasing levels of chloroquine restored expression to some extent, complete restoration was not achieved.

EXAMPLE 8: Transfection of COS-7 cells with RGD-polylysine peptides.

The methodology of Example 2 was repeated using COS-7 cells. In this experiment, different ratios of the K16 RGD peptide DNA were used with and without 100  $\mu$ m chloroquine, with the results shown in Figure 7. It can be seen that the optimum ratio was 37.5:1 in both the absence and presence of chloroquine.

10

EXAMPLE 9: Transfection of endothelial cells with RGD-polylysine peptides.

This was a repeat of Example 8 using ECV 304 endothelial cells. The results are shown in Figure 8.

15

EXAMPLE 10: Transfection of endothelial cells comparing K10 and K16 RGD peptides with lipofectamine.

20

ECV 304 endothelial cells were transfected with the optimised ratio of K16 RGD peptide-DNA complex (37.5:1) derived from Example 9. In addition, a range of ratios of K10 RGD peptide-DNA complex were also investigated, as well as transfection using lipofectamine. The results are shown in Figure 9, with the optimised K16 RGD-DNA complex beingz the most efficient.

CLAIMS

1. A composition comprising DNA associated with a polycation moiety wherein the polycation moiety is itself coupled to an integrin receptor binding moiety.  
5
2. A composition as claimed in claim 1 wherein the integrin receptor binding moiety is a peptide.
- 10 3. A composition as claimed in claim 2 wherein the peptide comprises the sequence RGD.
4. A composition as claimed in claim 2 or claim 3 wherein the peptide is cyclic.  
15
5. A composition as claimed in claim 4 wherein the peptide comprises at least two cysteine residues capable of forming a disulphide bond.
- 20 6. A composition as claimed in claim 5 wherein the peptide comprises the sequence GGCRGDMFGC.
7. A composition as claimed in any one of claims 1 to 6 wherein the polycation moiety consists of polylysine.  
25
8. A composition as claimed in any one of claims 1 to 7 wherein the polycation moiety consists of 10-16 cation residues.
- 30 9. A composition as claimed in claim 8 wherein the polycation moiety consists of 16 cation residues.
10. A composition as claimed in claim 9 wherein the ratio of polycation moiety to DNA is 37.5:1.

11. A composition as claimed in any one of claims 1 to 8 wherein the DNA is double stranded.
12. A composition as claimed in claim 11 wherein the DNA comprises one or more complete gene coding sequences.  
5
13. A composition as claimed in claim 12 wherein the DNA includes the coding sequence of the cystic fibrosis gene.
- 10 14. A DNA binding composition comprising a polycation moiety coupled to an integrin receptor binding moiety.
15. A composition as claimed in claim 14 modified by any one or more of the features of claims 2 to 9.  
15
16. A method of preparing a composition as defined in any one of claims 1 to 10 which comprises the step of bringing together a composition as defined in claim 14 or claim 15 with DNA.
- 20 17. A method as claimed in claim 16 wherein the DNA is as defined in any one of claims 11 to 13.
- 25 18. The use of a composition as defined in claim 14 or claim 15 in the preparation of a composition as defined in any one of claims 1 to 13.
- 30 19. A method for expressing DNA in a host cell which comprises the step of bringing together the host cell and a composition as defined in any one of claims 1 to 13.
20. A method as claimed in claim 19 wherein the host cell is a mammalian cell.

21. The use of a composition as defined in any one of claims 1 to 15 in the preparation of a medicament for the treatment or prophylaxis of a condition caused by a genetic deficiency or modification.

5

22. A pharmaceutical formulation comprising a composition as defined in any one of claims 1 to 13 together with one or more pharmaceutically acceptable carrier, diluent and/or excipient.

10

23. A composition as defined in any one of claims 1 to 15 for use in medicine.

15

24. A method for the treatment or prophylaxis of a condition caused by a genetic deficiency or modification comprising administering to a subject a composition as defined in any one of claims 1 to 13 or a pharmaceutical formulation as defined in claim 22.

20

25. A method for preparing a composition as defined in claim 14 or claim 15 comprising the steps of:

(a) synthesising an integrin receptor binding peptide coupled to a polylysine moiety;

25

(b) subjecting the peptide to a cyclisation step;

(c) purifying the mixture of peptides obtained by ion-exchange chromatography;

30

(d) assaying fractions for presence of integrin receptor binding peptide;

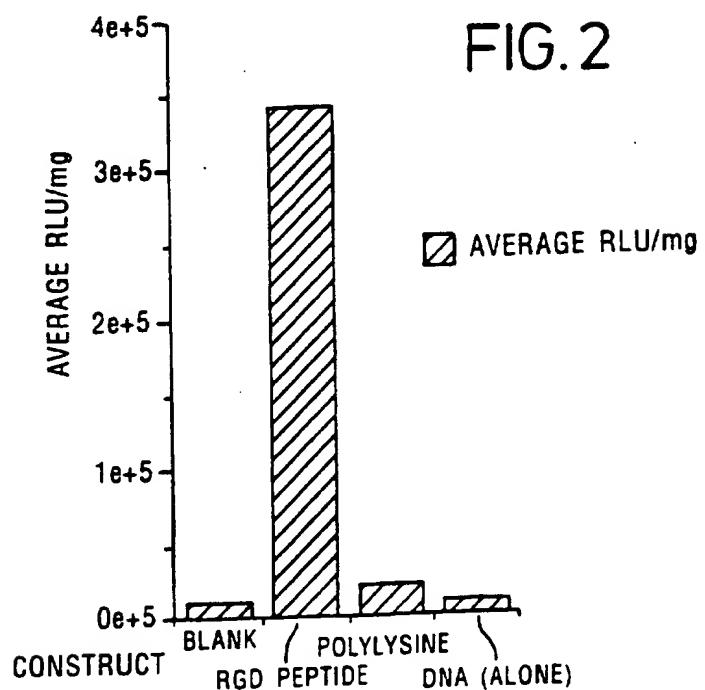
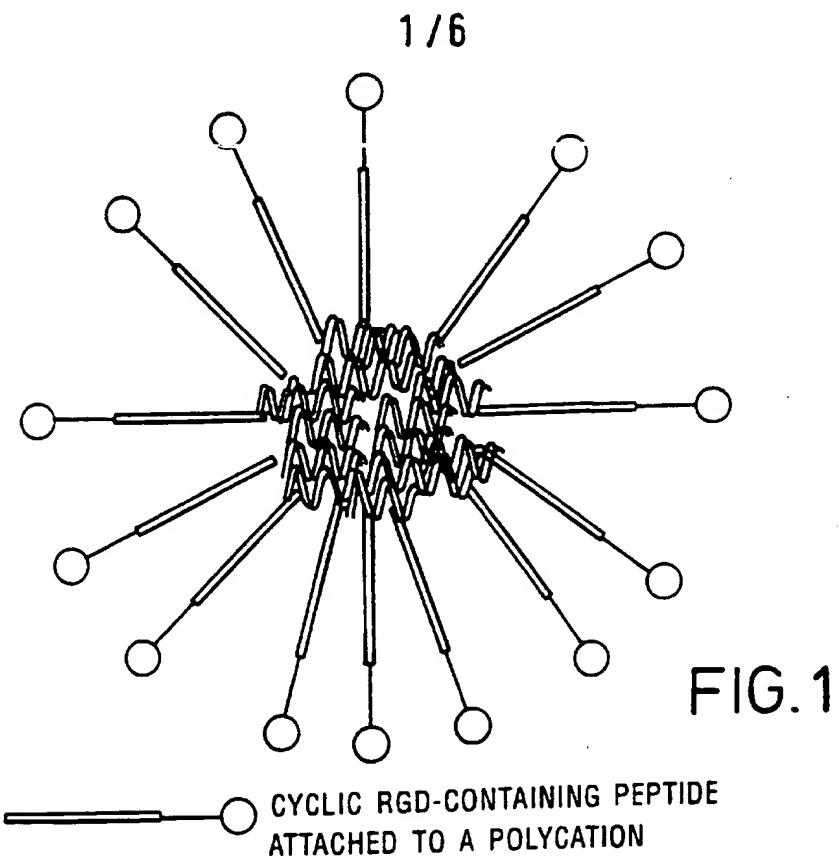
(e) desalting fractions containing integrin

receptor binding peptide; and

(f) further desalting the integrin receptor binding peptide containing fractions using reverse phase chromatography.

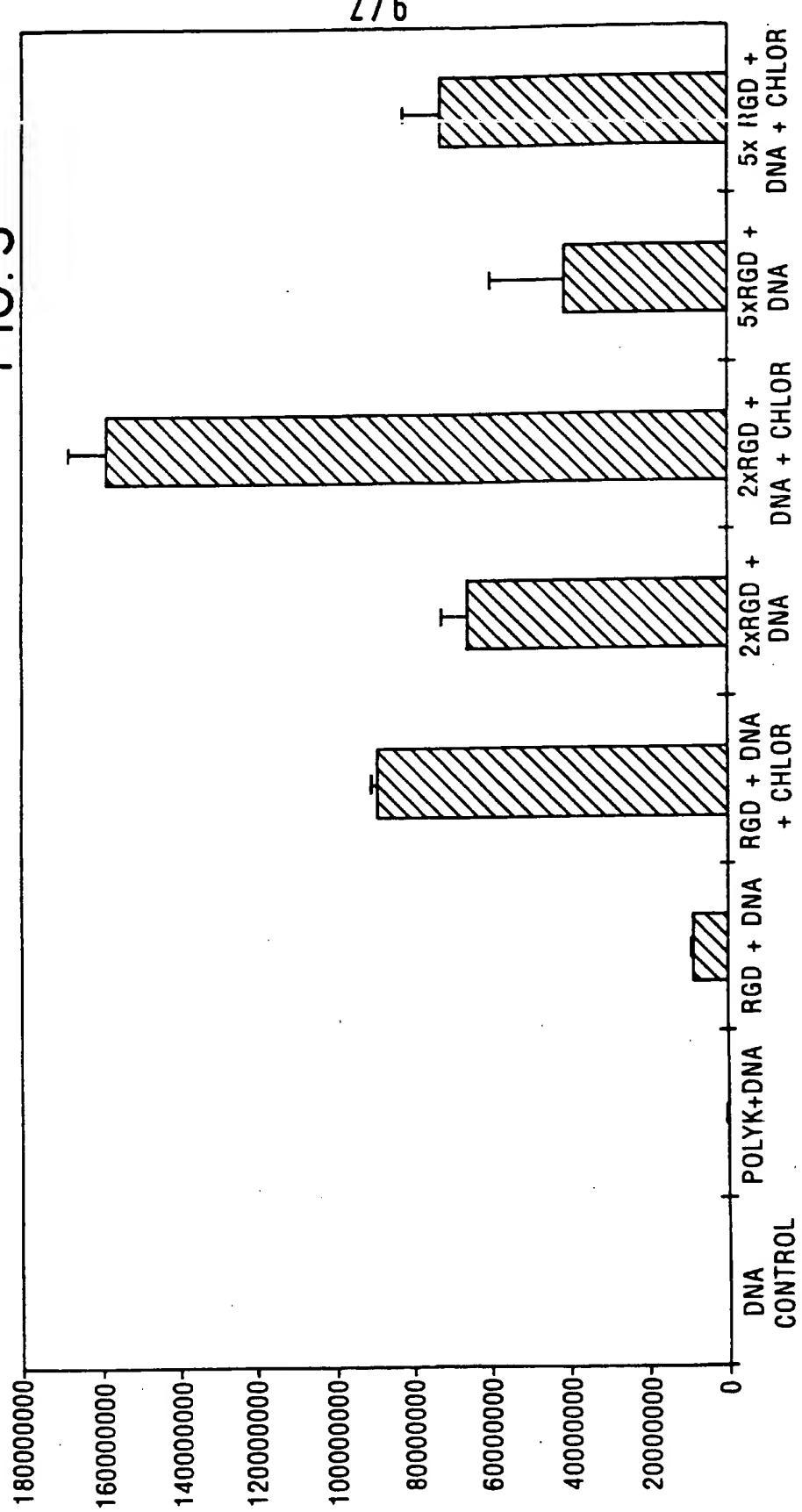
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26. A composition according to any of claims 1 to 13, further comprising a factor which reduces endosomal degradative activity.



SHEET 2 CHART 1

FIG. 3



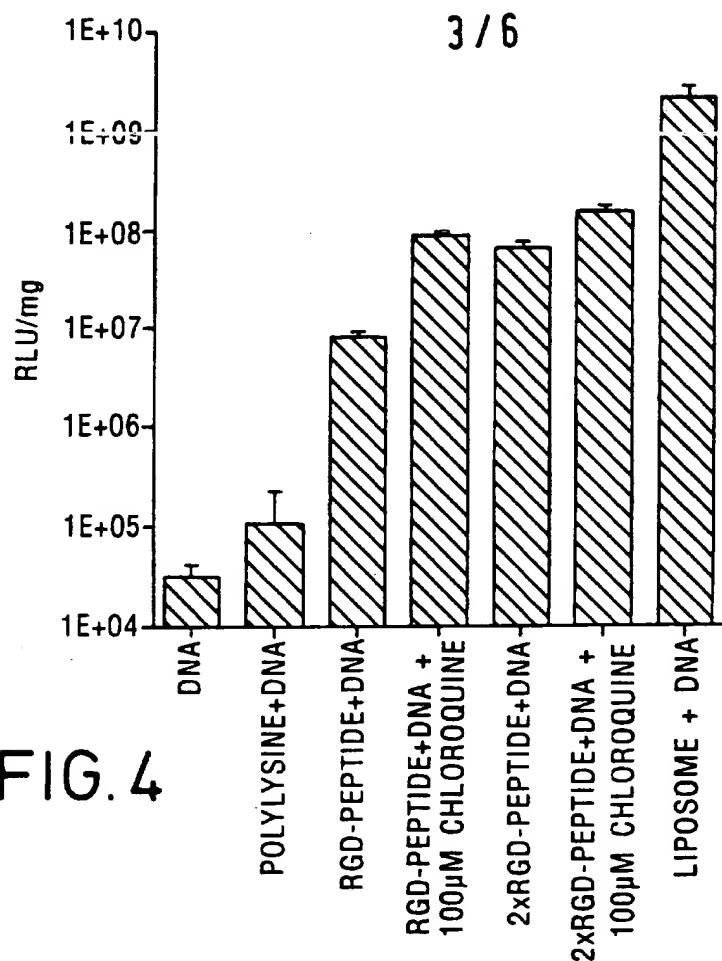


FIG. 4

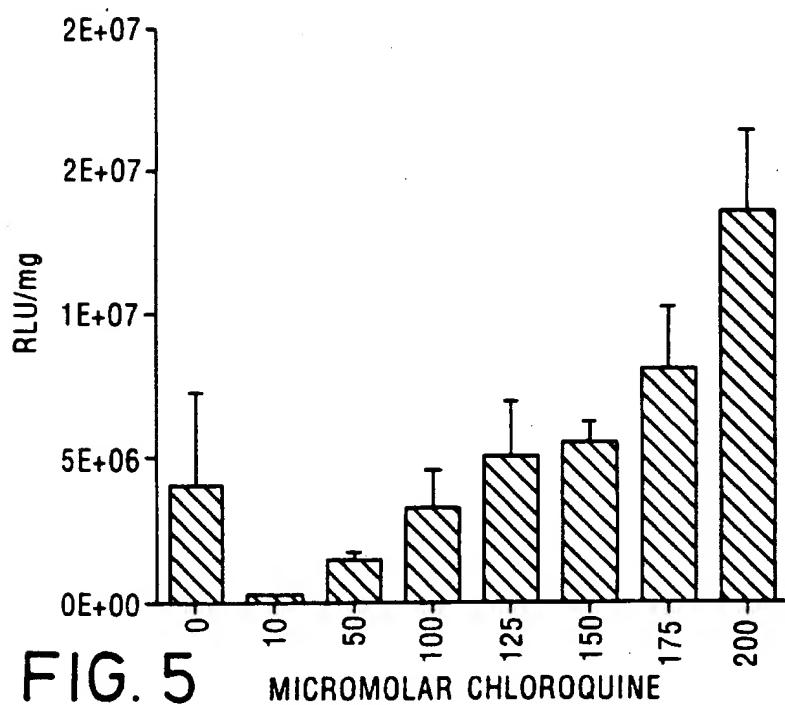
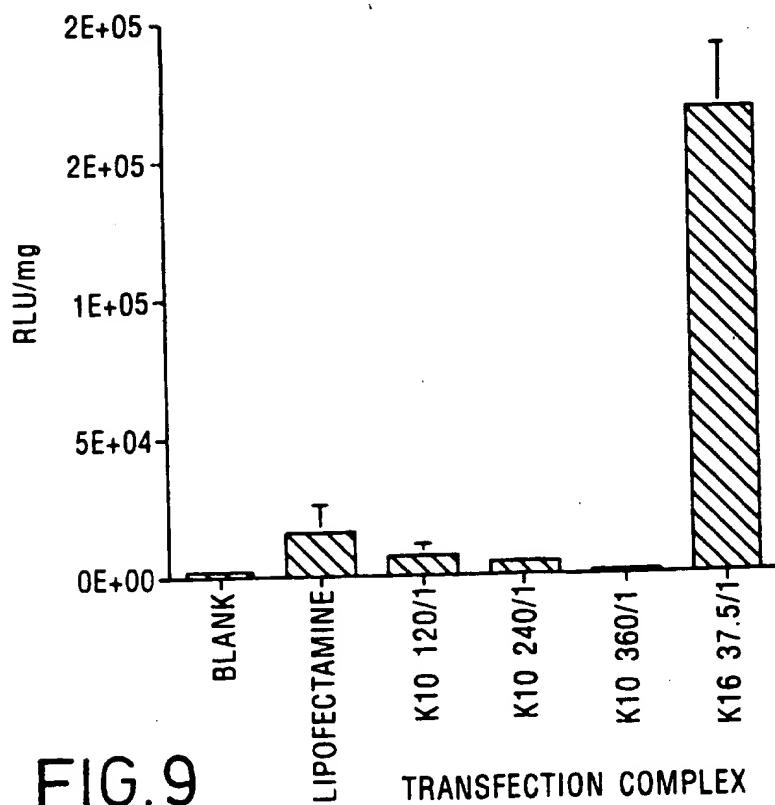
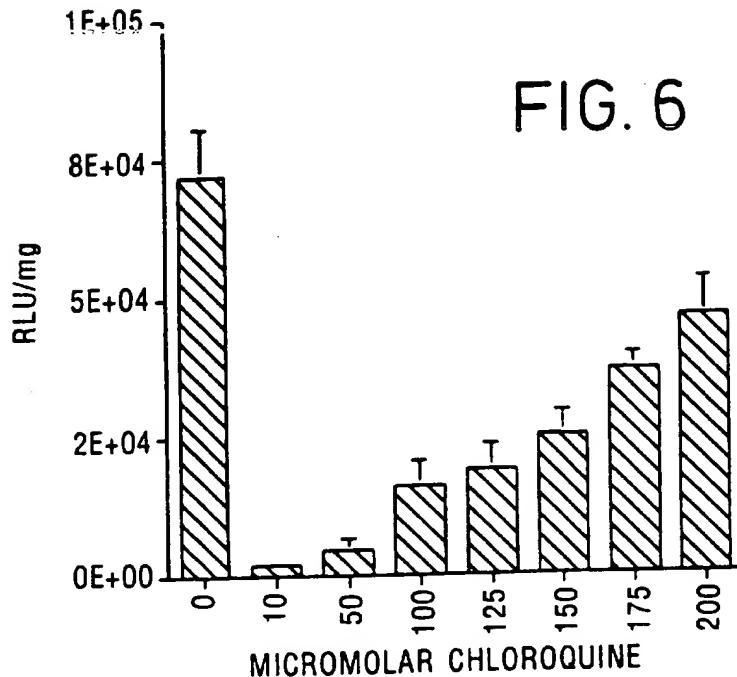


FIG. 5

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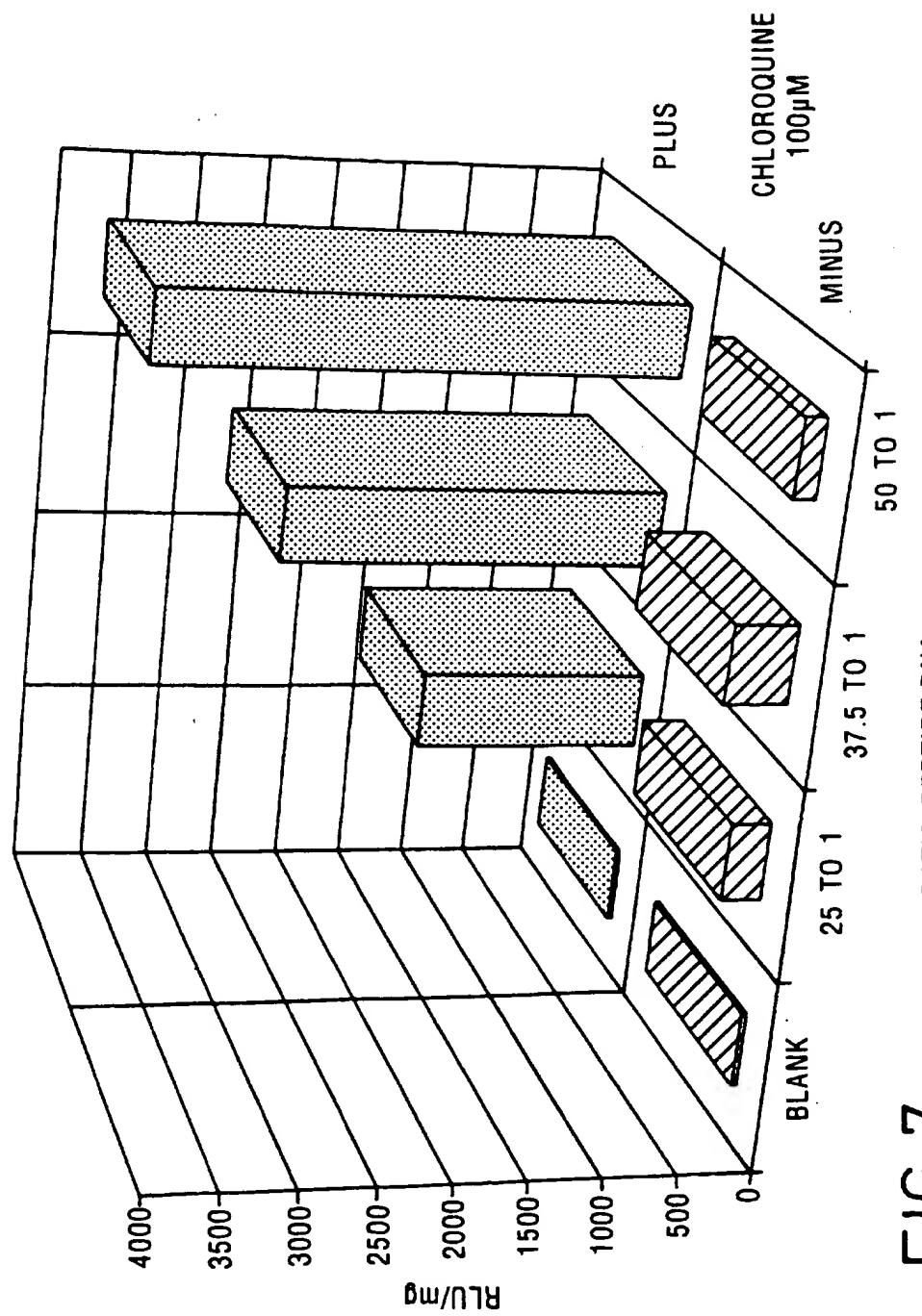


FIG. 7

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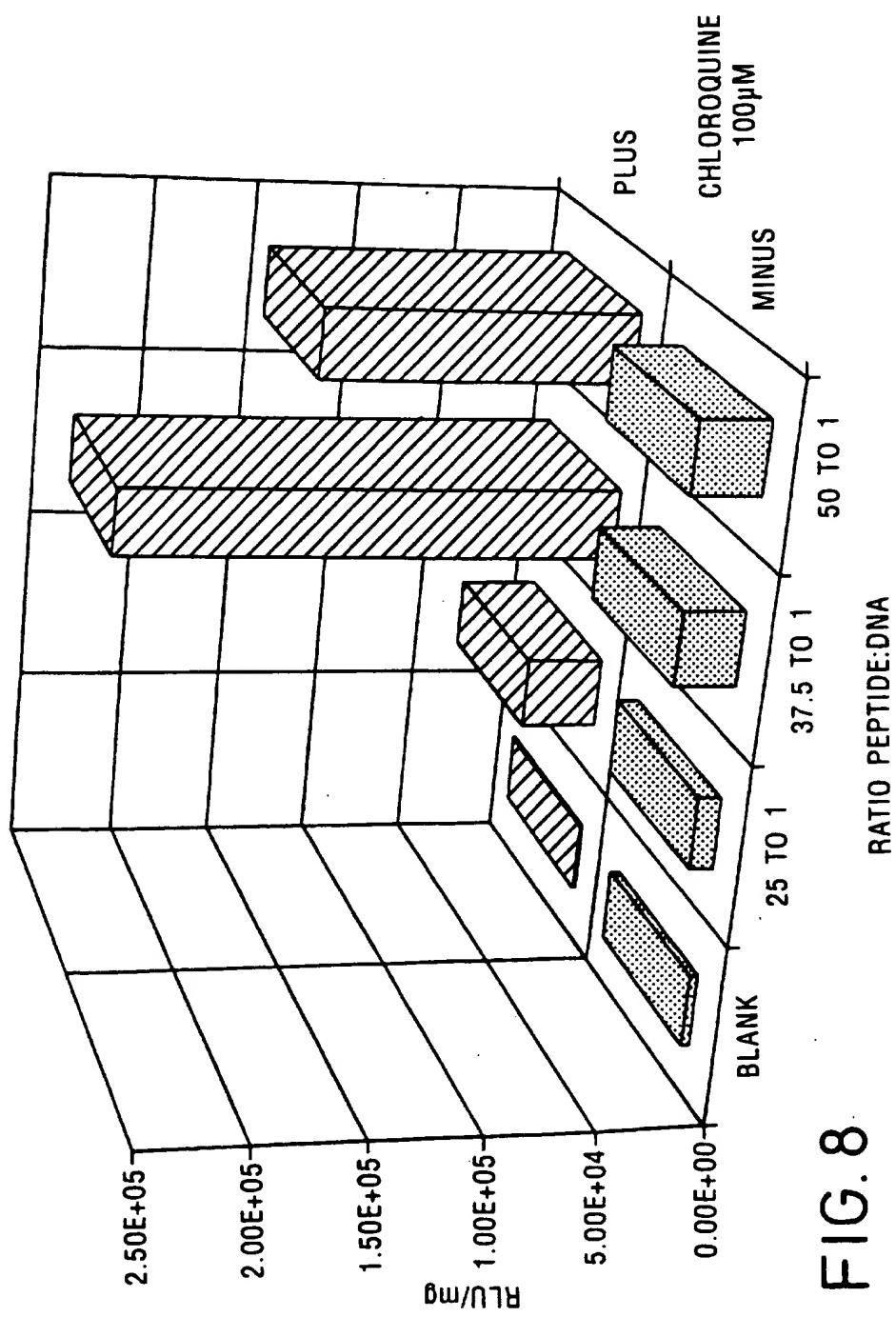


FIG. 8

# INTERNATIONAL SEARCH REPORT

Int'l Application No  
PCT/GB 95/02706

**CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 A61K47/48 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>COTTEN M. ET AL 'Receptor-mediated transport of DNA into eukaryotic cells', METHODS ENZYML., 1993, VOL. 217, PART H, PAGE(S) 618-644, USA XP 000562730</p> <p>see page 619</p> <p>see figure 1</p> <p>see page 622 - page 623</p> <p>---</p>	1-26
X	<p>J. BIOL. CHEM., 29-4-1994, VOL. 269, NO. 17, PAGE(S) 12468-1247, USA,</p> <p>HART S.L. ET AL 'Cell binding and internalization by filamentous phage displaying a cyclic Arg-Gly-Asp-containing peptide' cited in the application</p> <p>see abstract</p> <p>see page 12473, right column</p> <p>---</p> <p>-/-</p>	1-26

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
20 February 1996	05.03.96
Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patenttaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Dullaart, A

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International Application No

PCT/GB 95/02706

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